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Early bactericidal activity of ethambutol, pyrazinamide and the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater) in patients with pulmonary tuberculosis

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The early bactericidal activity (EBA) of ethambutol, pyrazinamide and the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater: Mer National) was evaluated in patients with pulmonary tuberculosis who were sputum-positive on microscopy for acid-fast bacilli.

Twenty-eight patients (mean age 33 years and weight 51 kg on average; range 40 - 59 kg) were studied. The fall in viable counts of *Mycobacterium tuberculosis* in sputum collections during the 2 days following the start of treatment was estimated from counts of colony-forming units (CFUs) of *M. tuberculosis* per ml of sputum cultured on selective 7H10 agar medium. The EBA for ethambutol determined in 9 patients was 0.245 ± 0.046, $\log_{10}$ CFU/ml sputum/day, that for pyrazinamide was 0.003 ± 0.014 $\log_{10}$ CFU/ml sputum/day and that for Rifater 0.558 ± 0.054 $\log_{10}$ CFU/ml sputum/day. The results obtained are similar to those reported in a previous study of the first 2 days of treatment, but in smaller numbers of patients, and confirm the moderate EBA of ethambutol while pyrazinamide is again shown to have very little EBA. Rifater has a marked EBA which may be due mainly to the action of isoniazid. This methodology may be valuable in the rapid evaluation of the bactericidal activity of new antituberculosis agents and the comparison of different dose sizes of agents of the same class.

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Following the initiation of antituberculosis therapy, there is a rapid fall in the number of viable tubercle bacilli in the sputum of patients with pulmonary tuberculosis.[1] When quantified by means of serial counts of colony-forming units (CFU) of *Mycobacterium tuberculosis* in sputum, significant differences in bactericidal action between different antituberculosis agents have been found during the first 2 days of treatment.[2] As most of the organisms killed during this period are probably extracellular, actively metabolising and in a logarithmic phase of multiplication, estimation of this early bactericidal activity (EBA) may provide an *in vivo* estimation of the bactericidal capacity of different antituberculosis drugs and a comparison of the activity of various dose sizes of agents of the same class. Comparisons between the EBA of rifabutin and rifampicin have been made in two more recent studies.[3,4]

In the first study of EBA, undertaken in Nairobi, 27 agents alone or in combination were studied, usually in groups of only 4 patients.[2] We report here a further confirmatory study of the EBA of ethambutol and pyrazinamide and of the fixed combination of isoniazid, rifampicin and pyrazinamide (Rifater; Mer National) in the dosages used during routine chemotherapy in South Africa.

Material and methods

Patients

Twenty-eight patients with previously untreated pulmonary tuberculosis whose sputum was positive for acid-fast bacilli on microscopy were studied. Patients were between 18 and 60 years of age (mean 33 years; median 44 years) and weighed 51 kg on average (range 40 - 59 kg).

In 25 patients (89%), multicavitary disease was present which in 24 (86%)involved an area larger than the right upper lobe. None of the patients suffered from other complicating medical conditions and none of the women was pregnant.

Patients were allocated consecutively to groups receiving daily doses of ethambutol 1.2 g, pyrazinamide 2 g, or fixed-combination tablets (Rifater) each containing isoniazid 80 mg, rifampicin 120 mg and pyrazinamide 250 mg, administered in a dose of 1 tablet for every 10 kg body weight. At the conclusion of the study, patients were placed on the treatment regimen currently recommended by the South African Tuberculosis Control Programme, i.e. isoniazid, rifampicin and pyrazinamide for 6 months.

A 16-hour collection of sputum was made between 16h00 on day 1 and 08h00 the next day (S1). After the first collection was complete, the first drug dose was administered at least 60 minutes before breakfast and this procedure was repeated twice more on subsequent days to give S2 and S3 sputum collections. This procedure is summarised in Fig. 1. Because of the slow onset of bactericidal activity in the case of pyrazinamide,[2] a third dose of the drug was given followed by an S4 sputum collection. Sputum specimens were sent by air courier to Pretoria for analysis in the laboratories of the South African National Tuberculosis Research Programme.

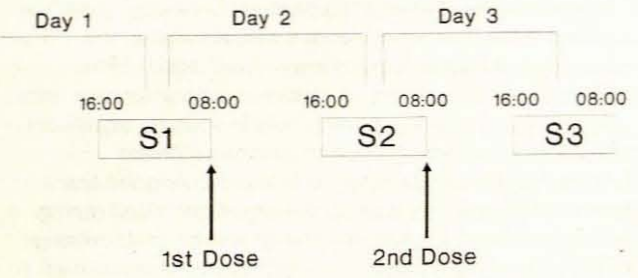

Fig. 1. Procedure for collection of sputum specimens S1, S2 and S3 and administration of antituberculosis agents.

Microbiological evaluation

Conventional smear, culture, sensitivity testing and CFU count were carried out as described previously[3] except that after 2 ml homogenised sputum were mixed with 3 ml 1:10 dithiothreitol (Sputolysin: Hoechst), 20 µl of the dilutions were set up without preliminary centrifugation on duplicate slopes of selective 7H10 medium in universal 28 ml screw-capped containers for CFU counting. Statistical evaluation was by means of *t*-tests.

The study protocol was approved by the Ethical Committee of the Medical Faculty of the University of Stellenbosch.

Results

A total of 35 patients was studied of whom 7 were excluded, 6 because they had negative or contaminated cultures and 1 because of initial drug resistance. The viable counts in the sputum collections from the remaining 28 patients obtained before chemotherapy (S1) and at daily intervals thereafter (S2 and S3) are given as means in Table I and illustrated for individual patients in Fig. 2. In patients given Rifater, there was only a slight fall on the first day of treatment (day 0 to day 1) but a large and abrupt fall the next day (day 1 to day 2), indicating a delay before the start of the bactericidal activity of the combination. The counts of patients given ethambutol declined slowly and to a similar extent between day 0 (S1) and day 1 (S2) and between day 1 and day 2 (S3). No decline in counts was seen in patients given pyrazinamide, even though treatment was extended by a third day. The error standard deviation from the analyses of variance (the patients × days of interaction) was greatest for the patients in the Rifater group and lowest in the pyrazinamide group.

The EBA was calculated as ($\log_{10}$ S1 count − $\log_{10}$ S3 count)/2 and is therefore the fall in $\log_{10}$ CFU/ml sputum/day. The mean EBA values found in the treatment groups of the present study are compared with those found in the Nairobi study[2] in Table II. The mean EBA for the combined tablet (Rifater) group was 0.558 $\log_{10}$ CFU/ml/day (± 0.054), the highest found; it can be compared with 0.685 $\log_{10}$ CFU/ml/day for the group in the Nairobi study that received

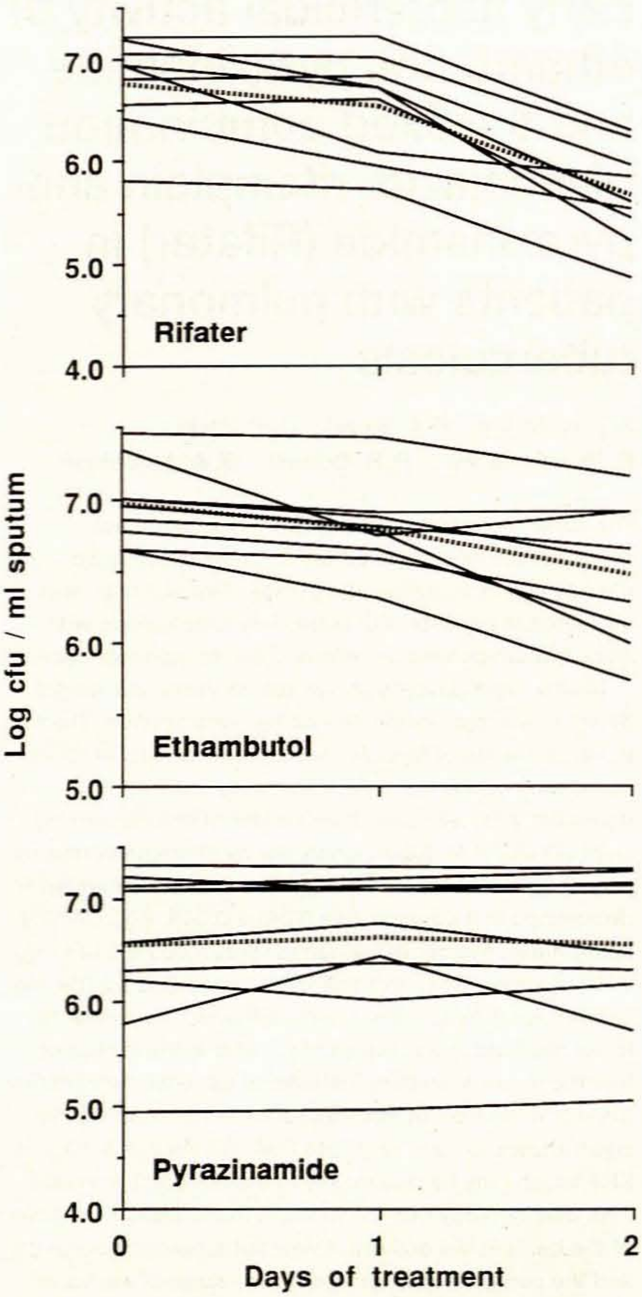

Fig. 2. Colony counts of *M. tuberculosis* in sputum in three groups of 9 patients each with pulmonary tuberculosis during the first 2 days of treatment with Rifater and with ethambutol and pyrazinamide. Interrupted lines represent mean values.

Table I. Counts of viable *M. tuberculosis* in sputum collections

		Viable count $\log_{10}$ CFU/ml sputum				Error	
Drug	No. of patients	S1	S2	S3	S4	Degrees of freedom	Standard deviation
Rifater	9	6.76	6.53	5.65	–	15	0.245
Ethambutol	9	6.96	6.78	6.47	–	16	0.181
Pyrazinamide	10	6.56	6.63	6.55	6.50	26	0.129

Table II. Early bactericidal activity of ethambutol, pyrazinamide and Rifater as estimated in Nairobi[2] and in the present study

		Fall in $\log_{10}$ CFU/ml sputum/day (No. of patients)			
			Present study		
Drug	Dose	Nairobi mean	Mean	95% confidence limits	
Rifater		0.685* (4) 0.598† (28)	0.558 (9)	0.682	0.435
Ethambutol	25 mg/kg	0.308 (4)	0.245 (9)	0.361	0.128
Pyrazinamide	2 g	0.044 (8)	0.004 (10)	0.032	−0.026

* Nairobi patients received separate daily doses of isoniazid 300 mg, rifampicin 12 mg/kg, pyrazinamide 2 g and streptomycin 1 g.
† Mean value for Nairobi patients given all combinations of isoniazid 300 mg with rifampicin 12 mg/kg and/or pyrazinamide 2 g.

the same three drugs plus streptomycin, though it must be remembered that streptomycin was thought to increase the EBA when given with pyrazinamide, and 0.598 $\log_{10}$ CFU/ml/day was the mean of all combinations of isoniazid with rifampicin and/or pyrazinamide. The mean EBA for ethambutol was lower at 0.245 $\log_{10}$ CFU/ml/day ($\pm$ 0.046), while pyrazinamide with an EBA of 0.003 $\log_{10}$ CFU/ml/day ($\pm$ 0.014) showed no apparent bactericidal activity during the 3-day treatment period. The estimates in the present study on larger groups of patients are thus similar to those found in the earlier Nairobi study. The standard deviations of the variations between patients in EBA were 0.161 for the combined tablet group, 0.152 for the ethambutol group and 0.041 for the pyrazinamide group. Similar results were obtained in a previous study.[4] The standard deviation for the group given isoniazid alone was 0.19 and the tendency for groups with a high EBA to have high standard deviations was also found.

No associations were found between the EBA and the S1 viable count, the radiographic severity of disease, degree of cavitation, or the weight, age or sex of the patient.

Discussion

The results obtained in the present study agreed well with those obtained in Nairobi.[2] Very low values for pyrazinamide were found in both studies, though pyrazinamide was found to kill bacilli slowly but steadily throughout the 14-day period of the Nairobi study.[2] Ethambutol had a moderately high EBA, similar to the Nairobi estimates for rifampicin and p-aminosalicylic acid. No direct comparison can be made for Rifater since this was not available at the time that the Nairobi study was done. However, in the Nairobi study, all combinations of isoniazid with other drugs gave values no higher than the particularly high EBA found with isoniazid alone, suggesting that other drugs given with isoniazid, except perhaps streptomycin, did not influence the EBA of combinations. The EBA of Rifater in our study was similar to the value for all combinations that did not include streptomycin, and only slightly lower than the value for the streptomycin-containing combination in the Nairobi study.

It is important to realise that during the first 2 days of treatment EBA is directed against bacilli in the sputum that arise from the very large bacterial population in cavity walls, distant from any phagocytic cells and therefore predominantly extracellular.[5] Those bacilli that are multiplying rapidly are also those that are killed most rapidly by bactericidal drugs and it is therefore their death that is measured by EBA. The EBA thus estimates the activity of a drug against rapidly dividing extracellular bacilli and is closely related to the conventional *in vitro* measure of bactericidal activity, viz. the fall in CFU counts during exposure of a culture in the logarithmic phase of growth to a drug. It can be used to compare the activities of different doses of the same drug or to compare closely related drugs, each tested at several dose levels.[3,4]

There are two fundamental measures of the activity of an antituberculosis drug: its ability to prevent the emergence of resistance to another drug and its sterilising activity.[6] The EBA is a measure of drug activity during the critical period when drug-resistant mutants are most likely to be selected from the large initial viable bacterial population. However, the ability of a drug to prevent the emergence of resistance is unlikely to be directly related to its EBA, since high bactericidal activity is not essential or even desirable for preventing resistance. Ability to prevent resistance might be better measured by the extent to which the EBA is maintained as the size of the drug dose is decreased, since this would indicate whether the drug is active throughout the different lesions within the lungs, despite variation in penetration into the lesions or in local drug activity.

While appropriate measurement of the EBA may indicate the value of a drug in preventing resistance, it does not measure its sterilising activity, which is the ability of a drug to kill persistent bacilli that are metabolically inactive and consequently survive drug action until the later stages of treatment. These bacilli are not necessarily extracellular and indeed, evidence from EBA studies with rifabutin[3,4] and from the known likelihood of a breakdown of immunity in AIDS patients to cause reactivation of dormant organisms, suggests that they are probably intracellular or at least closely associated with immune effector cells. Sterilising activity is measured by the relapse rate during follow-up after chemotherapy has been completed and also by the rate at which sputum cultures are converted at about 2 months after its start.[7] It is of course of much greater practical importance than the EBA since it determines the period that treatment must be given to obtain a satisfactorily low relapse rate. There is little correlation between the EBA and the sterilising activity of drugs.[5] Rifampicin and pyrazinamide are therefore the most effective sterilising drugs and the backbone of modern short-course regimens, yet pyrazinamide has a very low EBA and rifampicin only a moderate EBA. On the other hand, ethambutol has a moderate EBA, as again demonstrated in this study, but has no sterilising activity. It is therefore only of value in preventing the emergence of drug resistance and not in shortening the duration of treatment.

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